IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Confirmation No.: 9165
de Silanes *et al.*	Art Unit: 1644
Application No.: 10/690,639	Examiner: Yunsoo Kim
Filed: October 23, 2003	Atty. Docket: 2399.0010001/JAG/LAV
For: **Pharmaceutical Composition of $F(ab')_2$ Antibody Fragments**	

Declaration of Jorge F. Paniagua-Solis Under 37 C.F.R. § 1.132

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Sir:

I, Jorge F. Paniagua-Solis, declare and state as follows:

1. I received my education at Chemistry Faculty at Universidad Nacional Autónoma de México. A copy of my curriculum vitae is attached as EXHIBIT 1.

2. I am currently employed at Laboratorios Silanes S.A. de C.V. ("Laboratorios Silanes"), where I hold the position of Research and Development Director. My work involves the development of antibody and antibody fragment pharmaceutical compositions for use as anti-venoms against a variety of venomous animals, including spiders, snakes and scorpions.

3. I am familiar with U.S. Appln. No. 10/690,639 ("the '639 appln.") and pending claims as well as the December 15, 2006, Office Action.

4. I have been told by attorneys for Laboratorios Silanes that the specification of a patent application describes the claimed invention while the claims establish the scope of the invention. I understand that currently pending claims 30-31,

36, 44-54, 61-63, 67 and 74-76 are directed to methods and pharmaceutical compositions comprising polyclonal $F(ab')_2$ antibody fragments capable of binding to a purified molecule or mixture of antigenic molecules found in the venom of scorpions. I understand that the claims have been rejected in the Office Action for, among other things, lack of enablement. I have been told by attorneys for Laboratorios Silanes that a specification is 'enabled' if it conveys to a person having ordinary skill in the art how to make and use the claimed invention. In my view, a "person of ordinary skill in the art" with respect to the above-identified patent application would be a person having at least post-doctoral level training and experience in the field of immunology. Furthermore, I understand that:

(a) the Examiner acknowledges on page 5 of the December15, 2006 Office Action that the claim element "pharmaceutical" is met by a dialyzing a purified antibody against distilled water (which is a pharmaceutically acceptable carrier); however,

(b) the Examiner alleges that the specification is not enabling for, among other things, a pharmaceutical composition comprising a polyclonal antibody $F(ab')_2$ antibody fragment that binds to a purified molecule or mixture of antigenic molecules found in the venom of a scorpion and is capable of neutralizing the venom.

5. In making this declaration, I demonstrate that one of ordinary skill in the field of immunology as of March 5, 2001, the effective filing date of the present application, given the teachings of the specification, could have prepared and administered in a reproducible manner, a pharmaceutical composition comprising

polyclonal $F(ab')_2$ antibody fragments capable of binding to a purified molecule or mixture of antigenic molecules found in the venom of a scorpion.

6. *In vivo* experiments were performed by me or under my direction, which demonstrate that pharmaceutical compositions comprising polyclonal $F(ab')_2$ antibody fragments prepared by the methods described in the '639 appln." are effective for neutralizing scorpion toxins in mice and humans.

7. Our data shows that pharmaceutical compositions comprising polyclonal $F(ab')_2$ antibody fragments prepared by the methods described in the specification of the '639 application are efficacious for prompt and sustained neutralization of multiple species of scorpions in mice and humans.

8. The antivenom compositions used in the experiments described herein were prepared using the following process described in the specification under aseptic conditions:

(a) a source of antibodies was generated, as described in the specification, from a horse (or combination of animals) that was immunized with a complex mixture of antigenic molecules comprising polyvalent venom from scorpions of the genus *Centruroides*, to stimulate the generation of specific antibodies against these toxins;

(b) the blood plasma of the immunized horse (or other animal) was obtained, diluted with depyrogenized water and treated with pepsin, followed by incubation and agitation of the plasma-pepsin mixture, as described in the specification;

(c) the resulting antibody digestion product, which included digested antibody fragments (*i.e.*, $F(ab')_2$ antibody fragments), was subjected to ammonium sulfate precipitation, as described in the specification;

(d) the supernatant produced by the ammonium sulfate precipitation was recovered through decantation and clarified by passing it through multiple tray filters, as described in the specification;

(e) the clarified supernatant was again subjected to ammonium sulfate precipitation to produce an $F(ab')_2$ antibody fragment suspension, as described in the specification;

(f) the $F(ab')_2$ antibody fragment suspension was clarified by centrifugation to produce a composition comprising a paste of $F(ab')_2$ antibody fragments separated from the supernatant layer, as described in the specification;

(g) the $F(ab')_2$ antibody fragment paste was subjected to dialysis or ultrafiltration, as described in the specification, to remove salts and low molecular weight components resulting in a solution that contained $F(ab')_2$ antibody fragments specific against scorpion venom, and was substantially pure and free of pyrogen;

(i) the soluble fraction of the dialyzed product containing the $F(ab')_2$ antibody fragments was separated and formulated with pharmaceutical excipients suitable for injection, as described in the specification;

(j) the $F(ab')_2$ antibody fragment pharmaceutical composition was dosified in pyrogen free flasks and lyophilized, as described in the specification.

In Vivo Experiments in Mice

9. In one set of *in vivo* experiments, $F(ab')_2$ antibody fragments made by the method described above were tested for effectiveness in neutralizing the poison of the *C. sculpturatus* scorpion in mice. In these experiments, a mixture of three median lethal doses of scorpion poison with different antivenom dilutions were incubated for one hour at room temperature. Following the incubation, groups of five female mice (18-20 g) were intravenously inoculated with the different dilutions. The survival percentage was tabulated 24 hours after the inoculation, and the median lethal dose of the *C. sculpturatus* poison neutralized by the $F(ab')_2$ antibody fragment composition was calculated using the Spearman-Kärber method. The LD_{50} of *C. sculpturatus* was determined by administering the poison to mice at different doses, and calculating percent survival at 24 hours. The LD_{50} of the scorpion toxin was calculated to be 12.7µg, which was then used to assess the neutralizing ability of the $F(ab')_2$ antibody fragment antivenom composition in mice.

10. The LD_{50} dose of scorpion venom was mixed with $F(ab')_2$ antibody fragment antivenom, incubated and then intravenously administered to mice. The survival percentage was tabulated after 24 hours. The end time point of 24 hours is no more than would typically be expected when measuring the efficacy of an antivenom in mice. The survival rate for mice that received $F(ab')_2$ antivenom dilutions of 1.25 and 1.75 was 100% after 24 hours. The survival rate for mice that received an $F(ab')_2$ antivenom dilution of 2.5 was 20%, and the survival rate for mice that received $F(ab')_2$ antivenom dilutions of 3.5 and 5.0 was 0%. The median lethal dose (LD_{50}) of the *C. sculpturatus* poison neutralized by the $F(ab')_2$ antibody fragment composition was

calculated to be 173.9 LD_{50}/vial. The Mexican Pharmacopeia requires a median lethal dose of at least 150 LD_{50}/vial. Thus, our higher value of 173.9 LD_{50}/vial demonstrates very good neutralizing ability of *C. sculpturatus* venom by our $F(ab')_2$ antibody fragment composition.

11. In another set of *in vivo* experiments, the neutralization capacity of our $F(ab')_2$ antibody fragment composition against *T. pachyurus pocock* scorpion venom was characterized in mice. The *T. pachyurus* scorpion is classified as a moderately toxic scorpion venom based on an LD_{50} of 4.8 mg/kg. The poisoning signs observed in mice coincide with the effects induced by poisons of other scorpions in the *Buthidae* family, both in experimental animals and humans. Some signs of poisoning observed in mice include sialorrhea, difficulty breathing, generalized perspiration, staggering (ataxia) and behavioral alterations (excitability, drowsiness) during the first 15-30 minutes after inoculation.

13. The $F(ab')_2$ antibody fragment composition used in these experiments was prepared according to the method described above by horse immunization with the venom of *C. limpidus limpidus*, *C. noxius noxius* and *C. limpidus tecomanus* scorpions. The lyophilized $F(ab')_2$ antibody fragment composition was reconstituted with 5 ml of water suitable for injection.

14. Mice of the Swiss Webster strain, with a weight of 18 to 20 g were used in these toxicity tests. The lethal dose 50% (LD_{50}) of scorpion toxin for the inoculated mice was determined using the Spearman-Karber Method. For the initial toxicological determination, variable doses of venom were diluted in 500 µl of phosphate buffer

(PBS), adjusted to pH 7.2, and administered to groups of four mice per dose. Mortality was determined over a period of 48 hours. In order to identify systematic poisoning (sialorrhea, tachypnea, hyperhydrosis, etc.), groups of ten mice were inoculated. The first group received the injection subcutaneously in the right ball of the foot with 1.0 LD_{50} of the poison diluted in 50 µl PBS, and the second group received the injection intraperitoneally with 1.0 LD_{50} of the poison diluted in 500 µl of PBS. The mice were observed for 24 hours at 15, 30 and 60 minutes, and at 3 and 24 hours. Control groups were inoculated with PBS. The intraperitoneal LD_{50} in mice was 4.8 µg/kg, with 95% confidence limits of 4.4 to 5.2 mg/kg. The 100% lethal dose (LD_{100}), defined as the poison dose that caused the death of 100% of the inoculated mice, was 5.3 mg/kg.

15. The neutralization experiments were performed with poison and $F(ab')_2$ antibody fragment antivenom pre-incubation. Variable doses of $F(ab')_2$ antibody fragment antivenom and a fixed dose of poison (1.5 LD_{50}) were mixed and incubated for 30 minutes. These mixtures were later intraperitoneally administered to groups of four mice for each dose. The survivors were counted after 48 hours to determine the effective dose 50% (ED_{50}), defined as the dose which prevents death in 50% of the mouse population. The end time point of 48 hours is no more than would typically be expected when measuring the efficacy of an antivenom composition in mice. The ED_{50} is expressed in micrograms of poison neutralized by 1 ml of $F(ab')_2$ antibody fragment antivenom, and was obtained using the Spearman-Kärber method. The effective dose 100% (ED_{100}) and the survival times observed with different doses were also determined.

16. The ED_{50} of the $F(ab')_2$ antibody fragment antivenom was 330 µg of poison per ml of antivenom, with 95% confidence limits of 260 to 410 µg/ml. Thus, we

have demonstrated that the $F(ab')_2$ antibody fragment composition produced by the method above is effective for neutralizing scorpion venom *in vivo* in mice.

In Vivo Experiments in Humans

17. In another set of *in vivo* experiments, efficacy of $F(ab')_2$ antibody fragment composition produced using the above-described method was demonstrated in humans. The experiment involved the participation of 15 children who presented to hospitals in Arizona with systemic signs of scorpion envenomation. All the patients received intravenous midazolam sedation (standard of care) prior to enrollment in the study. Participants were then administered either placebo or $F(ab')_2$ antibody fragment antivenom composition. The antivenom was administered in a total volume of 50 ml by intravenous infusion over a minimum of 10 minutes. During the infusion, the patient was monitored for any changes in heart rate, blood pressure, respiratory distress, oxygen saturation and cutaneous manifestations of allergic reactions. If any signs suggestive of an acute allergic reaction were observed, infusion was to be stopped and appropriate interventions made.

18. One hour after infusion, blood samples were drawn to measure serum antigen levels, and physical assessments were repeated. Physical assessments were repeated 2 and 4 hours after infusion, and serum antigen levels were measured again 4 hours after infusion. The end time point of 4 hours is no more than would typically be expected when measuring the efficacy of an antivenom in humans. Clinically important components of the scorpion envenomation syndrome were divided into Pathological Agitation and Respiratory Compromise for separate documentation at each time point.

19. Components of Pathological Agitation included abnormal eye movements, thrashing of limbs, loss of ability to ambulate and presence of muscle fasiculations. Limb thrashing and abnormal eye movements were present in all 15 patients at baseline, despite prior use of benzodiazepine in all cases. These symptoms resolved within 4 hours for all patients who received the $F(ab')_2$ antibody fragment antivenom composition, but were still present for more than half of the children in the placebo group after four hours.

20. Components of Respiratory Compromise included pulmonary edema, incoordinate ventilatory effort, upper airway compromise, hypoxemia and other signs of respiratory distress. Of these, all except the pulmonary edema contributed to the overall toxicity determination. Respiratory compromise affected 3 patients at baseline, two of whom were in the $F(ab')_2$ treatment group, and one control patient. Resolution of respiratory effects was apparent in all 3 affected cases by 2 hours after infusion.

21. The primary endpoint was resolution of clinically important signs of scorpion envenomation. Pathological agitation resolved in 50% of $F(ab')_2$ antibody fragment antivenom composition recipients within 1 hour, 87.5% within 2 hours, and 100% within 4 hours of the antivenom infusion. Among the control patients, 71% still suffered from agitation at the 4 hour time point. Overall, resolution of clinically important signs of scorpion envenomation occurred in 100% of all 8 patients treated with the $F(ab')_2$ antibody fragment antivenom composition within 4 hours of treatment. Only 1 of the patients who received placebo showed evidence of resolution of symptoms, but the child's large size may have contributed to this early spontaneous resolution of symptoms.

22. Venom and antivenom blood levels were determined from plasma samples taken at baseline, 1 and 4 hours using immunoassay (ELISA) for *Centruroides sculpturatus*. The mean venom levels at baseline were similar in the $F(ab')_2$ and placebo treatment groups. Mean venom levels dropped below detectable amounts within 1 hour in all $F(ab')_2$ antivenom recipients. In contrast, mean venom levels in placebo recipients slowly declined, but were still measurable at both 1 hour and 4 hours after baseline. This difference supports the conclusion that $F(ab')_2$ antivenom efficacy is a consequence of venom binding by the antivenom; and it indicates that the dose of antivenom administered was sufficient for the prompt and sustained neutralization of the quantity of venom injected.

23. This study demonstrated that resolution of the overall symptoms of scorpion envenomation occurred more rapidly in $F(ab')_2$ antivenom recipients than in controls. A clinical difference between the two groups was apparent by 1 hour post-treatment, and dramatic by the 2 hour time point, with complete resolution among $F(ab')_2$ antivenom recipients within 4 hours of study drug infusion. Among patients who received placebo, all but one remained significantly symptomatic at 4 hours.

24. In conclusion, the experiments described above demonstrate that $F(ab')_2$ antibody fragment antivenom pharmaceutical composition prepared according to the method described above, and in the '639 application, are effective for *in vivo* use, both in mice and humans with an extremely high rate of successful resolution of symptoms associated with envenomation in both species. Further, the data presented above also indicates that there is cross-reactivity between a nonspecific or polyvalent antipoison and the poisons of scorpions of the other species within the same family. More specifically,

the $F(ab')_2$ antivenoms produced against scorpions of the *Centruroides* genus were effective in neutralizing the toxins of a variety of scorpions in the *Buthidae* family.

25. I further declare that the above statements made of my own knowledge are true and the above statements based on information and belief obtained from the references and documents discussed are believed to be true. Additionally, I declare that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Title 18 United States Code Section 1001, and that willful false statements may jeopardize the validity of this application or any patent issuing thereon.

Respectfully submitted,

Jorge E. Paniagua-Solis

Date: June 15th, 2007

RESUMEN CURRICULAR

Jorge F. Paniagua- Solís

Jorge Paniagua es Director de Investigación Inmunotecnologica del Grupo Silanes, S.A. de C.V. Es **Investigador Nacional Nivel I**. Sus funciones están encaminadas al desarrollo de Sistemas de Diagnóstico y Faboterápicos dentro de las áreas de: Inmunoterapia y diagnóstico molecular. Es responsable de diversos proyectos de Investigación y Desarrollo Tecnológico que se realizan con Instituciones Nacionales como: Instituto de Biotecnología/UNAM, Facultad de Medicina/UNAM, Instituto de Investigaciones Biomédicas/UNAM, CINVESTAV, la Universidad Autónoma de Morelos, Universidad Autónoma de Nuevo León, etc. y con Instituciones Extranjeras como la Universidad de Arizona, Texas A&M University y la Red de Centros para Intoxicaciones (Poison Control Centers). También se encarga de los proyectos de transferencia de Tecnología con diversas Empresas Biotecnológicas en Estados Unidos de Norteamérica, con la finalidad de incorporar tecnología a la empresa. Como consecuencia de esta actividad posee varias patentes nacionales e internacionales y otras se encuentran en trámite, en el área del empleo de los faboterápicos como formulaciones terapéuticas y de diagnóstico. Ha sido responsable de la creación de plataformas biotecnológicas de innovación en Laboratorios Silanes y en el Instituto Bioclon con las cuales, dichas empresas han fundamentado su plan estratégico y han sido pioneras en el éxito de la vinculación Academia-Industria. El modelo de gestión tecnológica desarrollado, en el Instituto Bioclon, fue reconocido con el **Premio Nacional de Tecnología 2005**. Bajo su coordinación se han obtenido las designaciones de drogas huérfanas por la FDA para los antivenenos y actualmente son los únicos medicamentos mexicanos que cuentan con autorización por la FDA para estudios clínicos en EEUU. Se espera obtener la aprobación de los mismos, por la FDA, en el 2008.

Nació en San Cristóbal de las Casas, Chiapas, México en 1964. Obtuvo la Licenciatura de Químico Farmacéutico Biólogo en la Facultad de Química, UNAM y la Maestría y Doctorado en Ciencias Biomédicas -Inmunología- en la Facultad de Medicina de la misma Universidad. Ha participado en diversos cursos internacionales y ha realizado estancias en el Instituto de Inmunología y Genética del Centro Alemán del Cáncer en Heidelberg, Alemania y en el Instituto Max Planck de Tubingen, Alemania. Fue becario del Gobierno Francés en la especialidad de Gestión de Biotecnología en la Escuela Superior de Comercio de París, Francia, en 1994.

Desde 1990 a 1997, se desempeñó en puestos de Investigación como: Jefe del Departamento de Investigación en Biológicos y Reactivos del Instituto Nacional de Higiene y como Investigador Asociado de la Unidad de Investigación Médica en Inmunoquímica del Instituto Mexicano del Seguro Social.

Profesor titular de Inmunología General y del Diplomado en Inmunología Básica y su Aplicación al Laboratorio, en la Facultad de Química de la UNAM desde 1994.

Tiene más de 40 publicaciones internacionales en revistas con arbitraje, 7 capítulos en libros y ha editado un libro. Ha participado en múltiples Congresos Internacionales.

Obtuvo el Premio **Nacional de la Juventud en 1989**, en el mismo año también recibió el Premio Anual de Investigación Médica del Instituto Syntex. Le otorgaron la **Medalla Gabino Barreda por sus estudios de Posgrado en 1992**. En el **2006**, recibió la medalla al mérito Sancristobalense "Dr. Manuel Velazco Suárez".

Pertenece a la Sociedad Mexicana de Inmunología, a la Sociedad Mexicana de Biotecnología y Bioingeniería, A.C., a la Sociedad Internacional de Toxinología, a la Asociación Farmacéutica Mexicana y al Colegio Nacional de QFB's.

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